Poster III-52

Computational and Experimental Analysis of the Molecular Mechanisms Mediating Actin Cytoskeleton Dynamics in Living Cells

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The actin cytoskeleton is a dense polymer assembly that continuously adapts its structure to accommodate many cell functions. For example, it is involved in forming the cell cortex, providing mechanical stability to the cell and thought to influence the organization of the plasma membrane into separate domains; in generating the forces required for cell polarization and migration; or in promoting endocytic, exocytic and intra-cellular transport processes. Our ultimate goal is to establish a quantitative model for how molecular effectors change the biochemical and mechanical properties of the actin cytoskeleton at the ultra-structural level in order to support all these functions. Besides our interest in understanding the basic principles of such multi-functional structures, the model will provide us with insights of the molecular mechanisms of normal and pathological cell behaviour, as related to actin cytoskeleton dynamics. Eventually, we will use the model to establish a screening framework for the discovery of drugs and genes that control actin-related cell responses.

Our work towards this goal is guided by the Manipulation-Measure-Model-and-Manipulation (the "4M") paradigm. We use advanced live cell microscopy and numerical models to measure and model the relationship between molecular action and cellular responses. The most important aspect of the 4M paradigm is the closed-loop investigation, starting and ending with molecular manipulations of the experimental cell system. By analyzing the dynamic responses to specific perturbations, we can step-by-step refine the numerical model. After each round of experiments the updated model is used to design the next round of perturbations predicted to further increase the model quality and its numerical stability. We hope that with this approach, adopted from reverse engineering, it will be possible to crack the complexity of a large molecular system such as the actin cytoskeleton. Our long term goal is to implement the 4M concept in a framework we term Computer Aided Experimental Design, where predictions on the cellular level will be combined with structural and functional genomics databases to identify the next best experiment.

In this poster we will illustrate how we apply the 4M paradigm to study the function of actin cytoskeleton dynamics in mediating directed cell protrusion. Cell protrusion is the first event of a recurring cycle of protrusion, adhesion, contraction, and de-adhesion driving cell migration. It is thought to be the result of spatially regulated actin turnover and flow, the latter generated by forces associated with actin polymerization and / or motor-induced contraction. We have developed a new quantitative Fluorescent Speckle Microscopy (FSM) to read out actin dynamics from live cell experiments. In FSM, the actin cytoskeleton has a speckled appearance where each speckle is a stochastic reporter of local polymer transport and turnover. Computational analysis of millions of speckles filmed over an extended period allows us to transform the random FSM signal into high-resolution maps of actin assembly, disassembly and transport. We will show how we use these maps to estimate the parameters of a continuum-mechanical model that relates the spatially and temporally distributed events of actin polymerization, contraction, and selective adhesion of the cytoskeleton to the substratum to cell protrusion. We will also illustrate how we are combining the continuum-mechanical description of actin dynamics with models at the ultra-structural level in order to link molecular events such as the binding or unbinding of an actin cross-linker to the macroscopically observable event of modulated protrusion.

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